Biofuel Component Concentrations and Yields of Switchgrass in South Central U.S. Environments

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ABSTRACT

Optimizing biofuel production and quality from switchgrass (*Panicum virgatum* L.) may require matching of ecotype and morphological type to environments, particularly in southern regions. Nine genotypes from four combinations of ecotype and morphological switchgrass type were harvested from 1998 to 2000 in five sites across Texas, Arkansas, and Louisiana that varied in latitude and precipitation. An additive main effects and multiplicative interaction (AMMI) method was used to evaluate genotype $\times$ environment interaction (G $\times$ E) patterns for traits important to biofuel production. Compared with upland genotypes across all site-years, lowland genotypes had greater lignocellulose yields (3.26 vs. 7.40 Mg ha^{-1}), greater removal rates of soil N (41 vs. 83 kg ha^{-1}) and P (6 vs. 12 kg ha^{-1}), greater concentrations of moisture (394 vs. 452 g kg^{-1}) and cellulose (388 vs. 394 g kg^{-1}), and lower concentrations of N (6.3 vs. 5.7 g kg^{-1}) and ash (48 vs. 40 g kg^{-1}). Compared with northern ecotypes, southern ecotypes had greater lignocellulose yields (4.95 vs. 6.85 Mg ha^{-1}), greater removal rates of soil N (60 vs. 76 kg ha^{-1}) and P (8 vs. 11 kg ha^{-1}), greater moisture concentrations (417 vs. 445 g kg^{-1}), and lower ash concentrations (45 vs. 40 g kg^{-1}). Lignocellulose yield paralleled dry matter yield (DMY) patterns. Switchgrass biofuel production efforts in the south-central USA should focus on improving DMY of southern lowland genotypes to maximize lignocellulose yields, but management factors may be more effective in optimizing moisture, ash, and mineral concentrations for combustion.

S WITCHGRASS is widely adapted across the USA (Moser and Vogel, 1995) and has potential as a biofuel for cofiring with coal. Specific target concentrations of chemical constituents for optimal energy generation from switchgrass are currently unclear because biomass conversion technology is at a very early stage of development (Vogel and Jung, 2001). In general, combustion biofuels should contain a high concentration of lignocellulose as the primary energy-producing substrate with low accompanying concentrations of water, ash, and N (Hohenstein and Wright, 1994).

Morphology and physiology affect lignocellulose concentration, and these functions are primarily determined by photoperiod and precipitation in switchgrass (Moser

and Vogel, 1995). Morphological type is designated as lowland (tall, coarse stems, adapted to poor drainage) and upland (short, fine stems, good drought tolerance), while physiological ecotype is determined by latitude of origin (broadly classified as northern or southern). Because of the strong response of maturation rate to photoperiod, northern ecotypes generally have lower cell wall concentration than southern ecotypes at the same stage of maturity (Buxton and Fales, 1994). Hopkins et al. (1995) reported that cell wall concentration and in vitro dry matter digestibility (IVDMD) of switchgrass grown in Iowa, Indiana, and Nebraska were relatively stable over environments, but that large G $\times$ E interactions existed for dry matter (DMY) and cell wall yield. Selection efforts focused on improving IVDMD for livestock have also produced lower lignin concentrations (Vogel and Jung, 2001), suggesting that selection in the opposite direction might lead to genotypes with improved combustion biofuel characteristics. Casler and Boe (2003) reported G $\times$ E interactions for ash concentration among switchgrass cultivars. The extent of potential G $\times$ E interactions for other biofuel qualities such as harvest moisture and mineral concentrations in switchgrass has not been reported.

Moisture is undesirable in combustion processes because additional energy is required to dry material before it will burn. Jenkins et al. (1998) stated that 650 g kg^{-1} of moisture is the upper limit for self-supporting combustion of biomass, with wet fuels burning less cleanly and requiring a supplemental fuel source to support combustion when moisture exceeds 500 g kg^{-1}. Lewandowski and Kicherer (1997) cited 230 g kg^{-1} of moisture as the critical maximum for efficient combustion and safe storage of grass biofuels.

Biofuel ash has several undesirable properties for combustion energy conversion processes, and some consider it to be the most limiting factor in those processes (Jenkins et al., 1998). Compared with coal, biofuel ash contains more alkali elements that contribute to slagging (formation of deposits on surfaces exposed to radiant heat) and fouling (formation of deposits on heat-recovery surfaces) in furnaces and boilers, agglomerization of fluidized beds, and corrosion of metal surfaces, all of which severely affect operation of power plants (Olanders and Steenari, 1995; Tillman, 2000; Werther et al., 2000). Heating value of wood fuels has been negatively correlated with ash concentration (Jenkins et al., 1998), with every 10 g kg^{-1} increase in ash concentration decreasing heating values by 0.2 MJ kg^{-1}. Tillman (2000)

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Abbreviations: AMMI, additive main effects and multiplicative interaction; ANOVA, analysis of variance; DMY, dry matter yield; G $\times$ E, genotype $\times$ environment interaction; IVDMD, in vitro dry matter digestibility; L, lowland morphological type; N, northern ecotype; S, southern ecotype; U, upland morphological type.

reported that high ash levels in switchgrass and straw impaired furnace function by increasing slag viscosity, while wood ash levels usually were not high enough to cause slagging. Grass biofuels generally contain three to five times more ash than wood (42–66 vs. 11–25 g kg^{-1}, respectively, Olanders and Steenari, 1995; Burvall, 1997; Obernberger et al., 1997).

Nitrogen is undesirable in combusted material because it contributes to emissions of air-polluting compounds (Lewandowski and Kicherer, 1997; Obernberger et al., 1997; Jenkins et al., 1998). Lewandoski and Kicherer (1997) cited 10 g N kg^{-1} as the critical maximum for combustion biofuels. Nitrogen removed from the cropping system must also eventually be replaced to support production.

Yields of switchgrass in the south-central region of the USA are often lower than in other regions despite the apparently favorable environment (mild to moderate winter, hot summer, and adequate annual rainfall). The poor yields in southern regions may be related to adaptation of ecotypes and morphological types to particular regions. In the Midwest and Central Plains regions, yields of upland genotypes are often comparable to or better than lowland yields (Lemus et al., 2002; Casler et al., 2004), while the latter usually outperform the former in southern states such as Texas, Alabama, and Virginia (Sanderson et al., 1996; 1999). Most available cultivars are upland morphological types (Moser and Vogel, 1995), and availability of cultivars of southern ecotypes is even more limited. 'Alamo', the commercial cultivar of southernmost origin (USDA, 1995), produced top yields in trials conducted in both northern (Lemus et al., 2002) and southern (Sanderson et al., 1996; 1999) regions. The success of Alamo suggests that southern lowland genotypes may be more suited for biomass production in the south-central region than northern upland genotypes. We conducted a trial with the objective of characterizing G × E interactions for chemical composition and yield of biofuel components among switchgrass genotypes with four different combinations of ecotype and morphological type in five locations selected to differ in latitude and the amount and distribution of precipitation across the south-central

USA. Dry matter yields and stand persistence are reported elsewhere (Cassida et al., 2005).

MATERIALS AND METHODS

Switchgrass germplasm was evaluated at five locations that varied in latitude and annual rainfall: College Station, Dallas, and Stephenville, TX; Hope, AR; and Clinton, LA (Table 1). Soil types at each site were College Station—Weswood silty clay loam (fine silty, mixed thermic Fluventic Ustochrept); Dallas—Houston black clay (fine, montmorillonitic thermic Udic Pellusterts); Stephenville–Windthorst fine sandy loam (fine, mixed thermic Udic Paleustalfs); Arkansas—Bowie fine sandy loam (fine-loamy, siliceous, thermic Fragic Paleudult); and Louisiana—Dexter silt loam (fine-silty, mixed thermic Ultic Hapludalf). Switchgrass genotypes were classified as upland or lowland by morphological type and as northern or southern according to latitude of origin within the south-central region. They included: Alamo, a southern lowland cultivar from south Texas; 'Caddo', a northern upland cultivar from Stillwater, OK; SL931, SL932, SL941, southern lowland synthetic lines from central and southern Texas; NL931, NL942, northern lowland synthetic lines from Oklahoma and southern Kansas; NU942, a northern upland synthetic line from Oklahoma and southern Kansas; and SU942, a southern upland synthetic line from central and southern Texas. All synthetic lines were developed at Oklahoma State University. Greenhouse-grown seedlings were transplanted into prepared seedbeds between 16 July and 7 Aug. 1997. All seedlings were grown at one site to minimize variability. Seedlings were planted in 53-cm rows on 30-cm centers, with six rows per plot (plot size 3.0 × 6.1 m) at all sites except Dallas, where seven rows were used (plot size 3.6 × 6.1 m). Stands in College Station and Dallas were irrigated once in late June of the 1998 harvest year (25 and 250 mm, respectively) because severe drought threatened loss of stands. Other sites were not irrigated after the establishment year. At Arkansas, plots were treated with 0.56 kg a.i. ha^{-1} of 2,4-D (triisopropanolamine salt of 2,4-dichlorophenoxyacetic acid) plus 0.15 kg a.i. ha^{-1} of picloram (triisopropanolamine salt of 4-amino-3,5,6-trichloropicolinic acid) on 6 Mar. 1998, and with 0.0043 kg a.i. ha^{-1} of metsulfuron [methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate] on 6 Apr. 1999 and 24 Apr. 2000 to control broadleaf weeds. Plots were treated with 2,4-D at 1.06 kg a.i. ha^{-1} on 21 Aug. 1999 at Louisiana and on 6 June 1998 at Dallas. No weed control was necessary at College Station or Stephenville.

Table 1. Environmental characteristics at Stephenville, Dallas, and College Station, TX; Hope, AR; and Clinton, LA from 1998 through 2000.

	Location					
	Stephenville	**Dallas**	**College Station**	**Arkansas**	**Louisiana**	**SE†**
April-September growth period						
Total moisture, mm yr^{-1} (normal)	304 (474)	351 (519)‡	280 (545)	490 (691)	539 (803)	57.6
Mean maximum daily air temperature, °C	31.9	31.7	33.3	30.7	31.1	0.4
Mean minimum daily air temperature, °C	17.6	19.6	17.3	17.3	19.7	0.3
Harvest dates§						
1998	22 Sept.	22 Sept.	8 Oct.	28 Oct.	14 Oct.	–
1999	14 Oct.	6 Oct.	8 Oct.	8 Nov.	15 Oct.	–
2000	20 Sept.	26 Oct.	27 Oct.	28 Oct.	20 Oct.	–
Mean day of year at harvest	271	280	286	304	288	6.1
Latitude	32°13′ N	32°58′ N	30°36′ N	33°40′ N	30°51′ N	–
Longitude	98°12′ W	97°16′ W	96°21′ W	93°35′ W	90°3′ W	–
Elevation, m	400	207	107	107	70	–

† SE reflects variation among sites and years.
‡ Stands at College Station and Dallas were irrigated once (25 and 250 mm, respectively) in late June of the 1998 harvest year because severe drought threatened loss of stands.
§ Harvest dates for each site–year were determined as the earliest dates where active plant growth had ceased and weather was favorable for harvest.

All stands were fertilized once per year within 4 to 6 wk of initiation of spring growth. Phosphorus and K were applied according to local soil-test recommendations in Arkansas and Louisiana but were not required at other locations. Nitrogen was applied at 150 kg ha^{-1} to all stands except at Arkansas, where 168 kg ha^{-1} was applied. The center two (six-row plots) or three (seven-row plots) rows of each plot were harvested once per year at a stubble height of 10 cm. Harvest was targeted to occur in late summer or autumn when the standing crop stopped initiating new tillers and leaves were no longer green. In Louisiana, upland entries were not harvested in 2000 because stands declined to negligible yields. Yields were recorded as zero for these entries. At harvest, subsamples were obtained, weighed, dried at 55°C, and weighed again to calculate dry matter concentration at harvest and plot DMY. Samples were ground through a 1-mm screen, and chemical composition was analyzed from replications 1 and 3 for 1998 to 2000 at each site. Cellulose and lignin components of cell wall were measured using the methods defined by Van Soest and Robertson (1980). Ash was measured by combustion (A.O.A.C, 1990). Total N and P concentrations were measured by a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Sample weight was 1.0 g, digestion media used was 5 g of 33:1:1 K_2SO_4:$CuSO_4$:TiO_2, and digestion was conducted for 2 h at 400°C with 17 mL of H_2SO_4. Phosphorus and N in the digestate were determined by semi-automated colorimetry (Hambleton, 1977) with a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, New York). Constituent yields for cellulose, lignin, and lignocellulose and nutrient removal rates for N and P were calculated as the product of DMY and component or mineral concentrations. Data for monthly total precipitation and average maximum and minimum air temperatures (Fig. 1) were collected from weather stations at each location.

Data were analyzed as a multisite, multiyear strip block arrangement of treatments, with appropriate error terms to test year and site effects in the analysis of variance (ANOVA) (SAS Inst., 2001). Within each site, the design was a randomized complete block with two replications. Comparisons of ecotype and morphological type groups were made by orthogonal contrasts: upland vs. lowland (U vs. L), northern vs. southern (N vs. S), northern upland vs. northern lowland (NU vs.
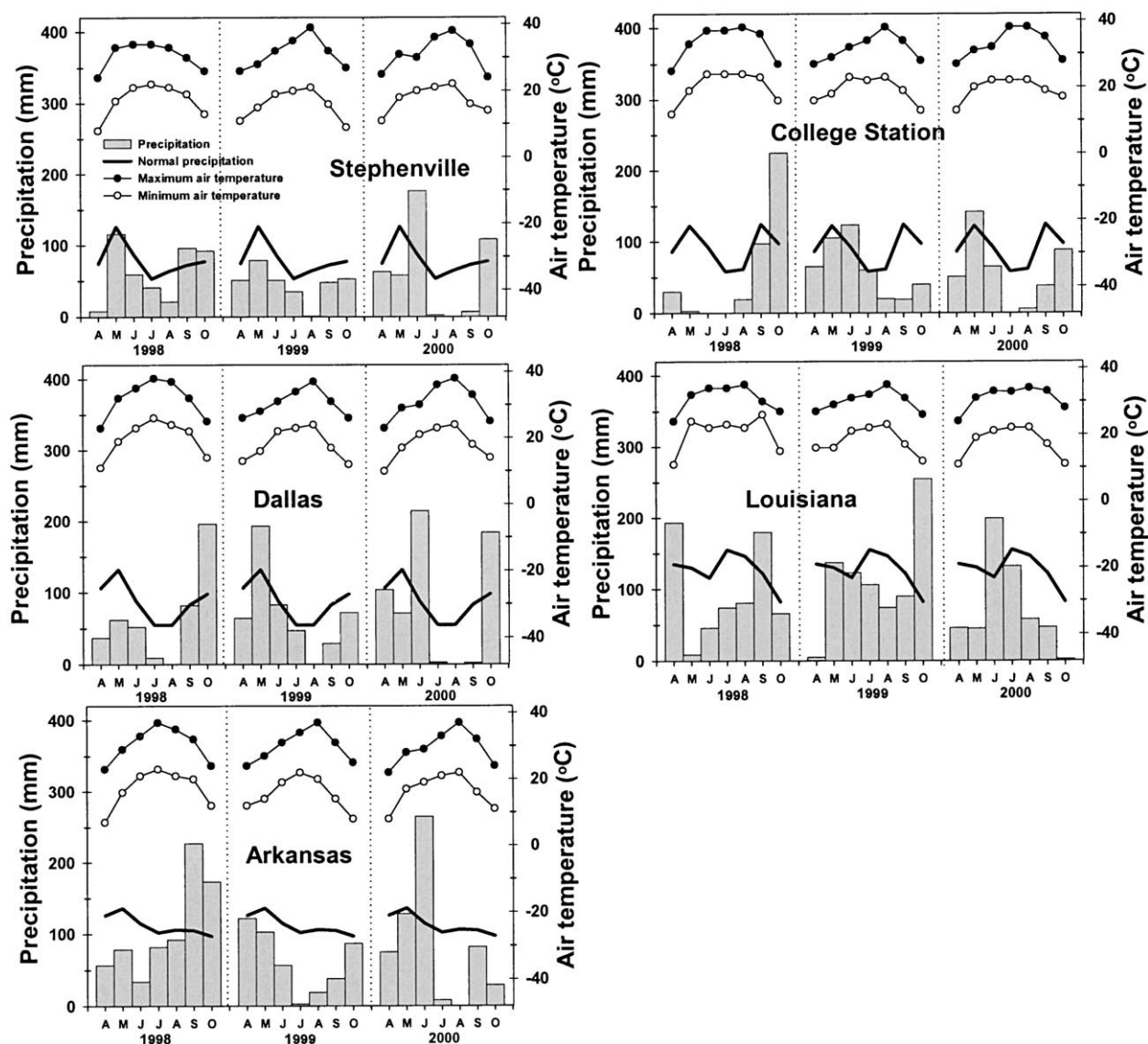

Fig. 1. Actual and long-term average monthly precipitation and average monthly maximum and minimum air temperatures for April to September, 1998 to 2000 at Stephenville, Dallas, and College Station, TX; Hope, AR; and Clinton, LA.

NL), and southern upland vs. southern lowland (SU vs. SL). The pattern of G × E interaction was evaluated by an AMMI method (Zobel et al., 1988; Zobel, 1992), with results presented as biplots. To allow estimability of AMMI terms for surviving genotypes, plant composition data for the dead upland genotypes at LA in 2000 were estimated by the method described by Snedecor and Cochran (1989). Influence of specific environmental variables on component concentrations and yields was evaluated on site–year (moisture availability, maximum and minimum temperature) or site (latitude) means by PROC REG (SAS Inst., 2001).

Confounding of compositional analysis with varying harvest dates across site–years was reduced by a regression method (Hopkins et al., 1995), in which all compositional components, DMY, and yield components were regressed on day of the year at harvest. Variables with significant relationships to day of harvest were adjusted to a common harvest date using the formula:

$$\text{Adjusted value} = \text{raw value} + [b \times (x - 287)] \quad [1]$$

where raw value is the unadjusted value, b is the slope for the corresponding regression (Fig. 2), x is the day of the year on which plots were harvested, and 287 is the day of the year corresponding to 15 October. The 15 October date was chosen because it was approximately in the center of the range of dates on which harvests were made across site-years. All mention of statistical significance throughout the text refers to $P < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

Harvest dates were targeted to occur when switchgrass stopped growing actively in autumn. This did not occur at uniform times across sites, resulting in confounding of stage of maturity and/or rate of senescence of biomass at harvest with harvest date. Harvest dates varied over a 49-d range (22 September to 8 November) among site–years. Biomass concentrations of cellulose, lignin, and lignocellulose increased linearly with calendar day at harvest (Fig. 2) and reported values for these constituents were therefore adjusted to the common harvest date. Biomass concentrations of water, ash, N, and P were not significantly related to harvest date and were not adjusted to the common date.

Cell Wall Concentration and Yield

Across site–years, contrasts (Table 2) showed that lowland genotypes had greater cellulose concentrations (394 vs. 388 g kg^{-1}) and greater yields of cellulose (6.03 vs. 2.63 Mg ha^{-1}), lignin (1.37 vs. 0.63 Mg ha^{-1}) and total lignocellulose (7.40 vs. 3.26 Mg ha^{-1}) than upland genotypes. Southern ecotypes had greater yields of cellulose (5.57 vs. 4.04 Mg ha^{-1}), lignin (1.29 vs. 0.92 Mg ha^{-1}), and total lignocellulose (6.85 vs. 4.95 Mg ha^{-1}) than northern ecotypes. Cellulose concentrations ranged from 339 to 463 g kg^{-1} and lignin concentrations ranged from 68 to 123 g kg^{-1}. These concentrations are greater than those reported for fall-harvested switchgrass biomass in Virginia, Texas (Sanderson and Wolf, 1995), Iowa (Lemus et al., 2002), South Dakota, and Wisconsin (Casler and Boe, 2003).

Results of AMMI analysis are presented as biplots, interpretation of which was summarized by Zobel et al.
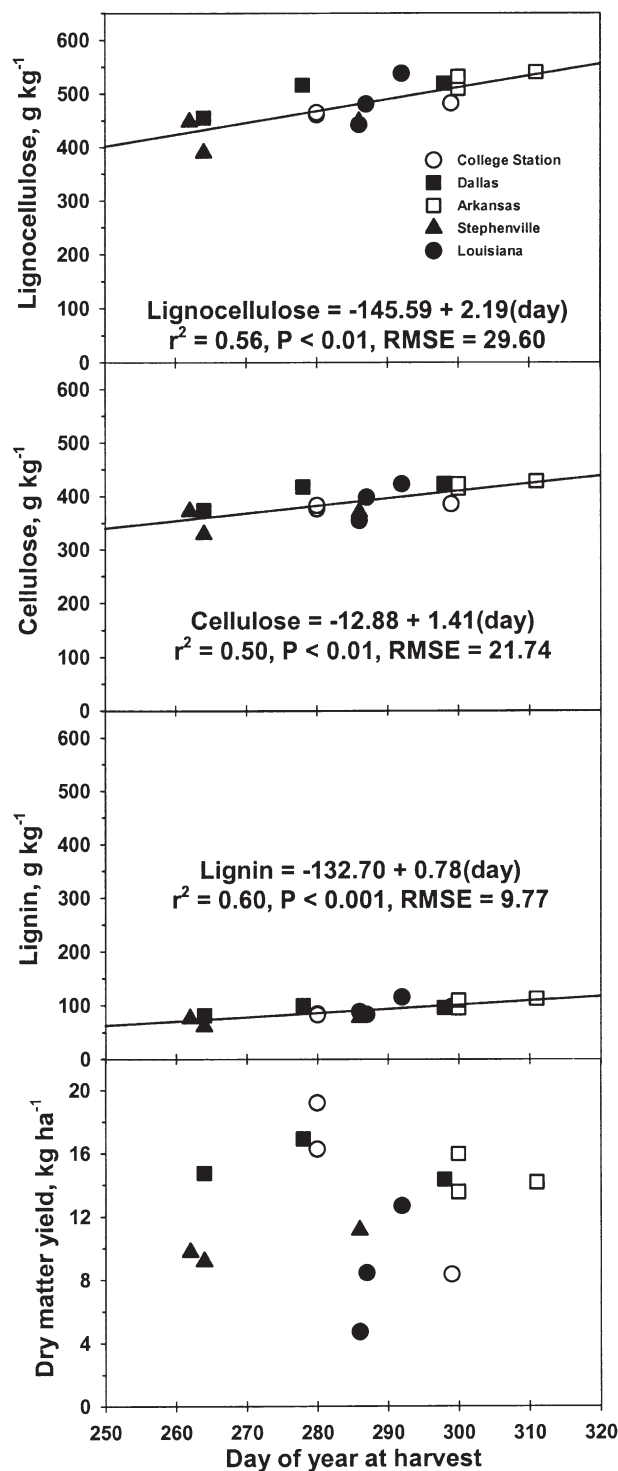

Fig. 2. **Relationship between day of year at harvest, and cellulose, lignin, and lignocellulose concentration, and dry matter yield in switchgrass harvested from 1998 to 2000 at Stephenville, Dallas, and College Station, TX; Hope, AR; and Clinton, LA.**

(1988). In brief, the sum of additive and multiplicative effects equals the value of any genotype–environment treatment mean. The additive main effect of genotype and environment equals the sum of genotype mean plus environment mean minus grand mean. For example, in Fig. 3, additive main effect for lignin concentration of

Table 2. Type III mean squares from analysis of variance for structural and chemical component yields and concentrations in nine switchgrass genotypes grown at five sites for 3 yr.

	df	Yield, Mg ha⁻¹					
		Dry matter	Lignocellulose	Cellulose	Lignin	N	P
Rep	1	7.1	1.0	0.8	0.02	486	13.1
Site	4	684.3**	201.3**	129.8**	8.03**	28 699**	267.1*
(E_A) Rep × site	4	22.0	5.7	3.4	0.29	1 127	23.9
Year	2	183.1***	56.6***	36.9***	2.26***	3 911	40.1
Year × site	8	138.1***	31.4***	21.3***	1.06***	8 826**	139.4**
(E_B) Rep × year × site	10	11.8	2.8	1.9	0.10	1 393	21.8
Genotype	8	572.9***	138.0***	92.3***	4.60***	13 778***	354.3***
U vs. L†	1	4 314.0***	1018.9***	684.7***	33.11***	105 696***	2663.2***
N vs. S	1	926.6***	236.7***	153.0***	9.10***	15 471**	484.1***
(E_C) Rep × genotype	8	5.5	1.5	1.0	0.05	861	12.7
Site × genotype	32	27.3***	6.4***	4.3***	0.22***	860*	14.1
Year × genotype	16	21.4***	6.4***	4.0***	0.27***	1 389**	23.2**
Year × site × genotype	64	13.2	3.6*	2.3*	0.15*	703	11.2
(E_D) Residual error	112	9.8	2.4	1.6	0.09	542	9.3
		Composition, g kg⁻¹					
		Cellulose	Lignin	Water	Ash	N	P
Rep	1	352	2.3	9 580	150	9.49	0.25
Site	4	11 189*	1313.8	320 157**	2400**	54.07**	2.64**
(E_A) Rep × site	4	803	260.7	5 889	66	1.60	0.14
Year	2	8 093***	844.4**	408 612***	102	123.03***	1.22***
Year × site	8	5 168***	1290.5***	17 342**	484**	39.46***	0.47***
(E_B) Rep × year × site	10	430	95.1	2 948	84	3.36	0.03
Genotype	8	1 205*	179.6	21 687**	630***	7.53	0.07
U vs. L†	1	3 349*	125.4	143 632***	3652***	25.79*	0.08
N vs. S	1	945	225.7	43 833**	2335***	22.52	0.02
(E_C) Rep × genotype	8	324	93.0	2 565	48	3.41	0.03
Site × genotype	32	400	229.2***	7 330**	106***	2.29	0.04
Year × genotype	16	575*	121.2	7 783**	84**	3.13*	0.03
Year × site × genotype	64	441*	99.3	5 716**	58**	2.26	0.03
(E_D) Residual error	112	300	92.1	3 400	35	1.71	0.03

* Significant at the 0.05 probability levels.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Contrasts: U vs. L–upland versus lowland morphological type, N vs. S–northern versus southern ecotype.

Alamo at Arkansas in Year 1 is 83.5 g kg⁻¹ (89.0 g kg⁻¹ mean for Alamo plus 85.2 g kg⁻¹ mean for A1 minus grand mean of 90.7 g kg⁻¹). The multiplicative G × E interaction effect equals genotype interaction score times environment interaction score (in the example, −3.7 g kg⁻¹, or 0.9 g kg⁻¹ PCA score for Alamo multiplied by –4.1 g kg⁻¹ PCA score for A1). Predicted treatment mean for Alamo at Arkansas in Year 1 is therefore 83.5 plus −3.7 g kg⁻¹, or 79.8 g kg⁻¹. From this it can be seen that G × E pairs that differ in mathematical sign of the interaction score must interact negatively (the multiplicative component will be negative and treatment means will thus be less than the additive main effect) and pairs with the same sign interact positively (a positive multiplicative component adds to the additive main effect). It can also be seen that genotype or environment points that are located close to the zero y axis will always have small multiplicative interaction components relative to points located farther from zero, and therefore points close to zero are more stable across a treatment factor than those farther away.

The AMMI analysis revealed one significant PCA axis for lignin concentration and two for cellulose concentration (Fig. 3). The wider spread of site–year points around the grand mean relative to genotype lignin or cellulose concentration means indicated that environment (site–year) factors had a greater impact on variation in biomass composition than did genotype, in agreement with Lemus et al. (2002). Genotypes differed in

stability of cell-wall components as reflected by the PCA interaction scores. Alamo was relatively stable (i.e., PCA score was close to zero) across environments for both cellulose and lignin concentration, NL942 was stable for cellulose but more variable for lignin, while SL931 and NL931 were stable for lignin but variable for cellulose.

Upland genotypes and Arkansas site–year means were clustered well apart from the rest of the data for lignin concentration, indicating a strong positive interaction between the upland genotype group and the Arkansas site. This pattern was not observed for cellulose concentration, nor did other sites display a pattern of G × E interactions for cell wall concentrations. Because Arkansas was the northernmost site and did not differ greatly from other sites in other environmental features, this result suggests that lignin concentration in upland genotypes depended more strongly on photoperiod or latitude than did cellulose concentration. Pattern significance in the second PCA axis for cellulose concentration was primarily attributable to a cluster of high cellulose concentrations in the Dallas environment and a high interaction score for Caddo across all environments.

Biplots of component yields of dry matter, cellulose, lignin, and combined lignocellulose (Fig. 4) revealed a strong environmental gradient pattern for each component. In general, the location of genotype and site–year points was similar for each component yield, indicating that DMY was relatively more important than cell wall
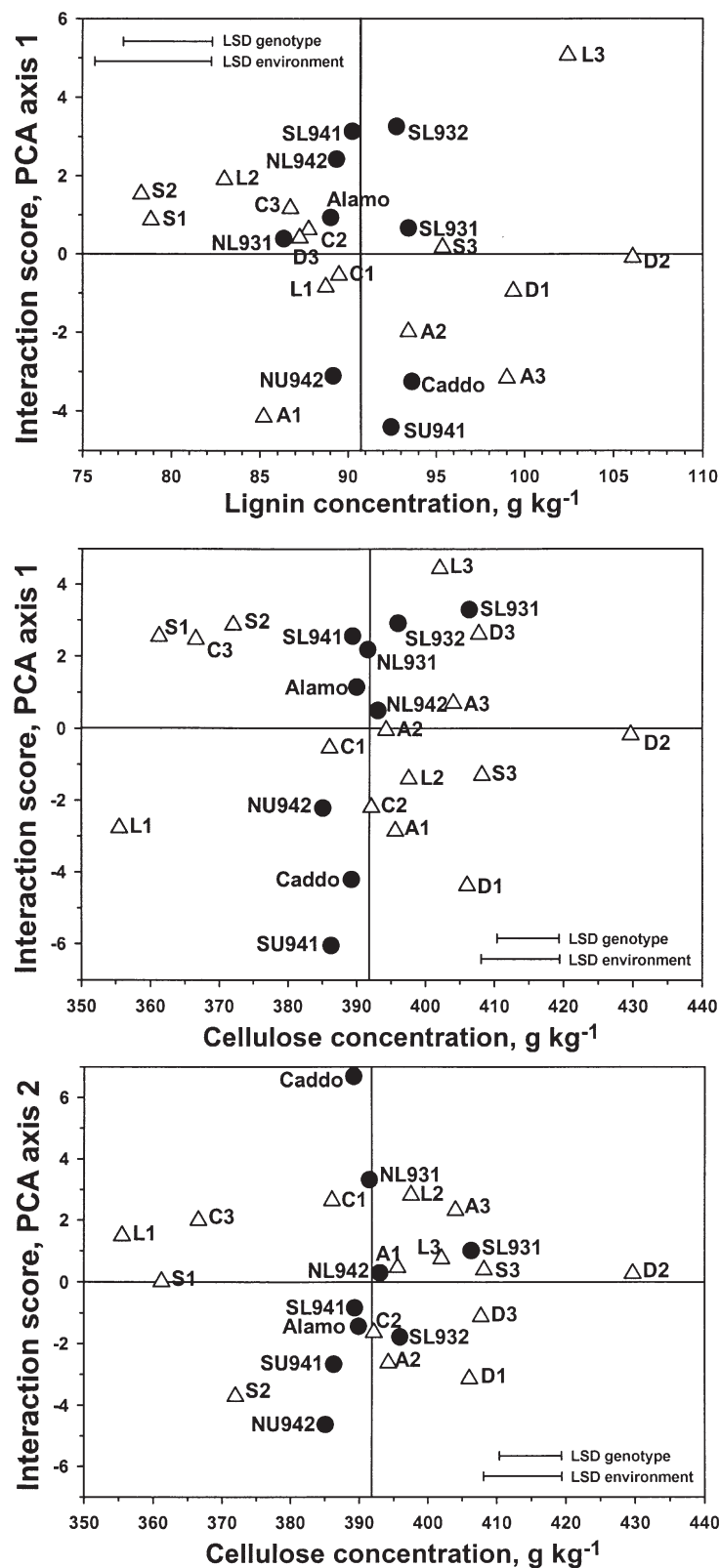
Fig. 3. Biplot of interaction principle component analysis (PCA) axis versus concentration of cellulose and lignin of nine switchgrass genotypes (solid circle) grown over 15 site–years (open triangle). Site-year labels consist of a site letter (A–Hope, AR; C–College Station, TX, D–Dallas, TX, L–Clinton, LA, and S–Stephenville, TX) plus a year number (1 = 1998, 2 = 1999, 3 = 2000). The vertical line is at the *x* axis mean.
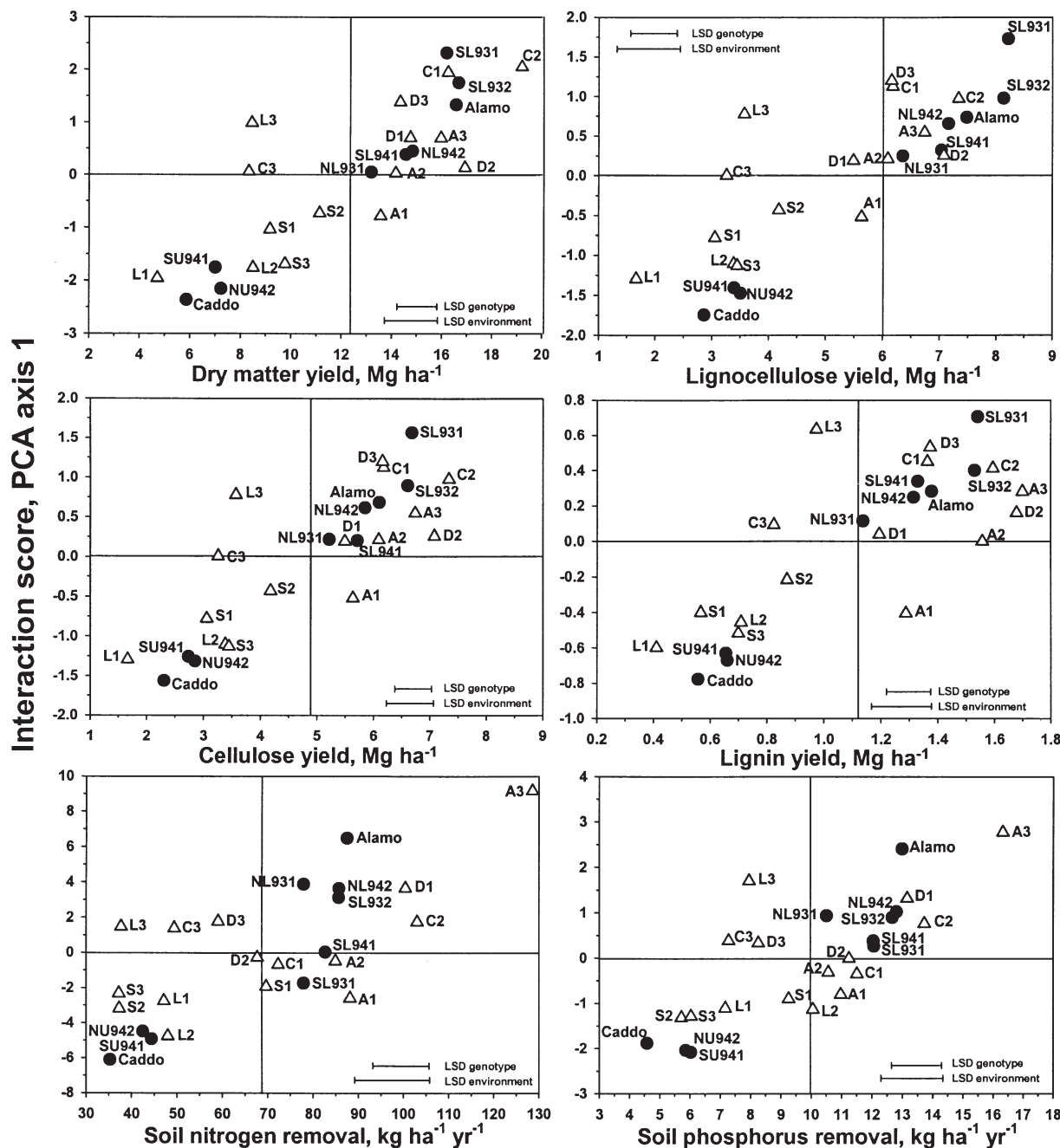
Fig. 4. Biplot of interaction principle component analysis (PCA) axis versus yield of dry matter, cellulose, lignin, or total lignocellulose and yearly removal of soil N and P for nine switchgrass genotypes (solid circle) grown over 15 site–years (open triangle). Site–year labels consist of a site letter (A–Hope, AR; C–College Station, TX, D–Dallas, TX, L–Clinton, LA, and S–Stephenville, TX) plus a year number (1 = 1998, 2 = 1999, 3 = 2000). The vertical line is at the *x* axis mean.

concentration in determining relative component yields. The lignocellulose yield biplot showed a more distinct separation of high-yielding SL genotypes than did the DMY biplot, but yields were not significantly different among the SL genotypes. These results indicate that even though lignocellulose yield represents the useful portion of yield for cofiring, DMY is an adequate measurement for the comparison of genotypes. Upland genotypes were both low-yielding and highly unstable for DMY, while Alamo, SL931, and SL932 were high-yielding and unstable. The greatest DMY stability across

these environments was exhibited by the two NL genotypes and SL941, and these genotypes also had acceptable average DMY.

The G × E interaction contribution to upland genotype yield was consistently positive at the dry Stephenville site, became increasingly negative with successive years at the wet Louisiana and Arkansas sites, and was always negative at Dallas and College Station. Conversely, lowland genotypes tended to show positive component yield interactions with higher moisture sites. These results are consistent with stand persistence data
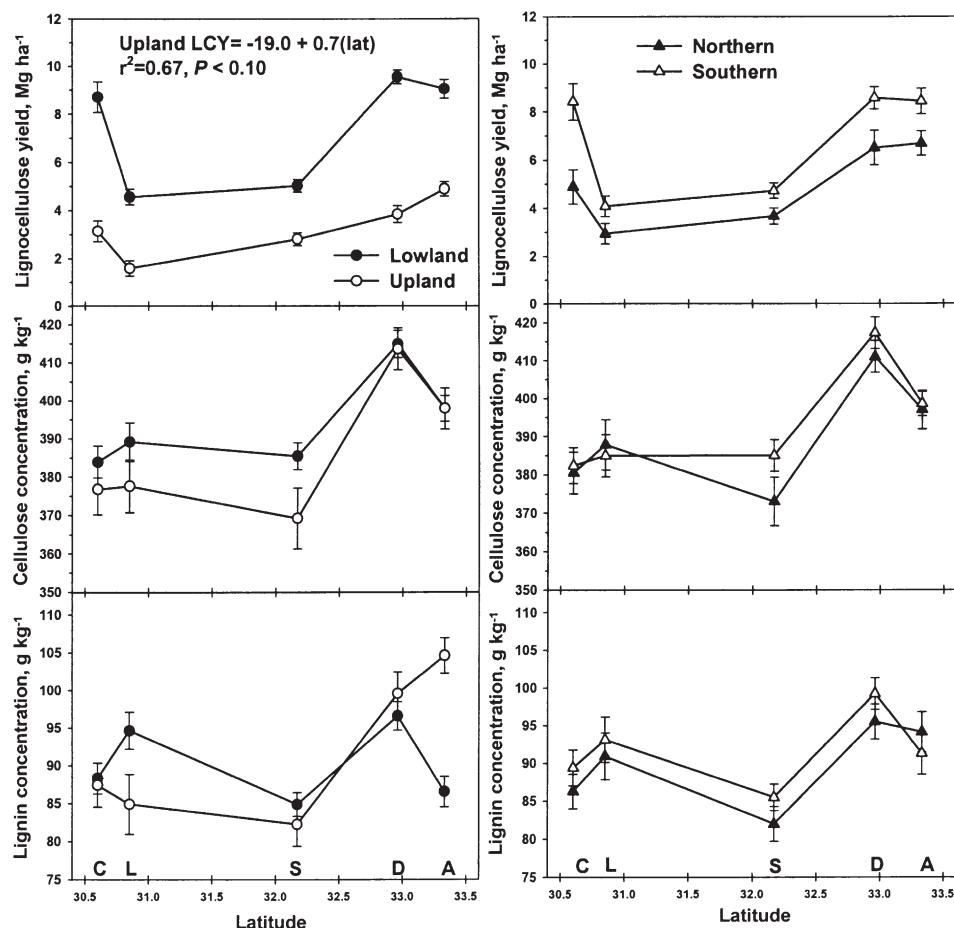
Fig. 5. Lignocellulose yield (LCY) and cellulose and lignin concentrations for lowland versus upland morphological types and northern versus southern ecotypes of switchgrass grown at five locations (A–Hope, AR; C–College Station, TX, D–Dallas, TX, L–Clinton, LA, and S–Stephenville, TX) differing in latitude. Regression equations indicate significant relationships ($P < 0.10$).

reported for this trial (Cassida et al., 2005), with expected adaptation of lowland and upland morphological types (Moser and Vogel, 1995), and suggest that lowland genotypes are more responsive to differences in moisture availability than are upland types.

Regression analysis was used in an effort to isolate environmental factors influencing results. Latitude was the only environmental regressor that was related to biofuel yields in any genotype group (Fig. 5). Lignocellulose yield tended to increase with latitude in upland genotypes but not in lowland genotypes. Lignin and cellulose concentrations were not affected by latitude in any genotype group, in contrast to results of Casler et al. (2004) at latitudes above 36°, who reported that holocellulose concentration increased with latitude in upland genotypes, but decreased sharply with latitude in lowland genotypes. This contrast could indicate that switchgrass response to latitude differs in the northern versus southern USA or simply that the range of latitudes (30°36′ to 33°40′) in our study was not large enough to allow detection of a linear effect. Also, differences in site moisture availability may have confounded responses to latitude for cellulose and lignin concentrations and lignocellulose yield. Drought conditions (site–year rainfall totals 17 to 73% below normal) during the primary growing period for switchgrass (April through

September) disturbed the normal west-to-east precipitation gradient expected on these sites (Table 1). In general, drought reduces grass cell wall concentrations through delayed stem development (Wilson, 1983), and this is consistent with the results observed for cell wall concentrations at the dry sites of College Station and Stephenville. However, irrigation at College Station and Dallas could have contributed to high cellulose concentration at Dallas and high lignocellulose yields at both sites if timing of moisture was more important than total amount. Regression based on total amount of moisture would not detect such a relationship. As in the biplot (Fig. 3), lignin concentration showed a strong interaction with site, being higher in lowland genotypes in Louisiana and in upland genotypes in Arkansas, two sites with similar rainfall but differing latitude.

Cellulose and lignin concentration and lignocellulose yield were always the same or greater for southern compared with northern ecotypes (Fig. 5). These results are consistent with the expected effects of latitude on daylength-sensitive grasses. At the same stage of maturity, cultivars adapted to northern latitudes (long photoperiods) generally have lower whole-plant cell wall concentration despite low leaf-to-stem ratios compared with southern-adapted cultivars (Buxton and Fales, 1994). Moving northern-adapted ecotypes southward to

shorter days tends to reduce cell wall concentration even further because of delayed flowering and increased leaf-to-stem ratios (Buxton and Fales, 1994). Lignocellulose yields reflected expected responses in DMY across ecotypes. Switchgrass ecotypes flower and go dormant earlier with concomitant reduction in total biomass production when grown south of their area of origin, and flower later with increased yield when moved north (Buxton and Fales, 1994; Sanderson et al., 1999; Vogel and Jung, 2001).

Biomass Moisture Concentration

Moisture concentration at harvest was greater for lowland than upland genotypes (451 vs. 394 g kg^{-1}) and for southern than for northern ecotypes (445 vs. 417 g kg^{-1}) (Table 2). Moisture concentration of biomass at harvest ranged from 124 to 644 g kg^{-1}, similar to that reported for single-cut fall harvests of switchgrass (Reynolds et al., 1996; Casler and Boe, 2003). Moisture data were characterized by complex interactions across all treatment levels, and the biplot (Fig. 6) did not show a clear environmental gradient pattern that would suggest a single overriding influence on moisture concentration. Since harvest date was also not related to moisture concentration, environmental factors may play the largest

role, possibly including autumn precipitation amounts and patterns, humidity, soil moisture holding capacity, and crop lodging characteristics that could influence wicking of soil moisture into the biomass.

The moisture levels of harvested biomass would be problematic to the bioenergy industry. Baling is a potential packaging method for transporting switchgrass from production site to electrical generators (Tillman, 2000), but large bales packaged at moisture levels greater than 180 g kg^{-1} are at risk of internal heating to temperatures sufficient to cause spontaneous combustion (Collins and Moore, 1995). Therefore, harvesting switchgrass for biofuel even as late as early November in the study region would likely require a drying period before safe baling, and November weather is rarely conducive to good field drying. An alternative method is to harvest biomass with a silage chopper and blow it into trucks for transport to the use site (Boylan et al., 2000), thus avoiding problems associated with bales. However, grass-based feedstocks should contain less than 230 g kg^{-1} moisture at the combustion point for efficient energy conversion (Lewandowski and Kicherer, 1997), and therefore nearly all of the biomass harvested in our trial would require additional drying before it would burn efficiently. Delaying harvest until mid to late winter has been successful at
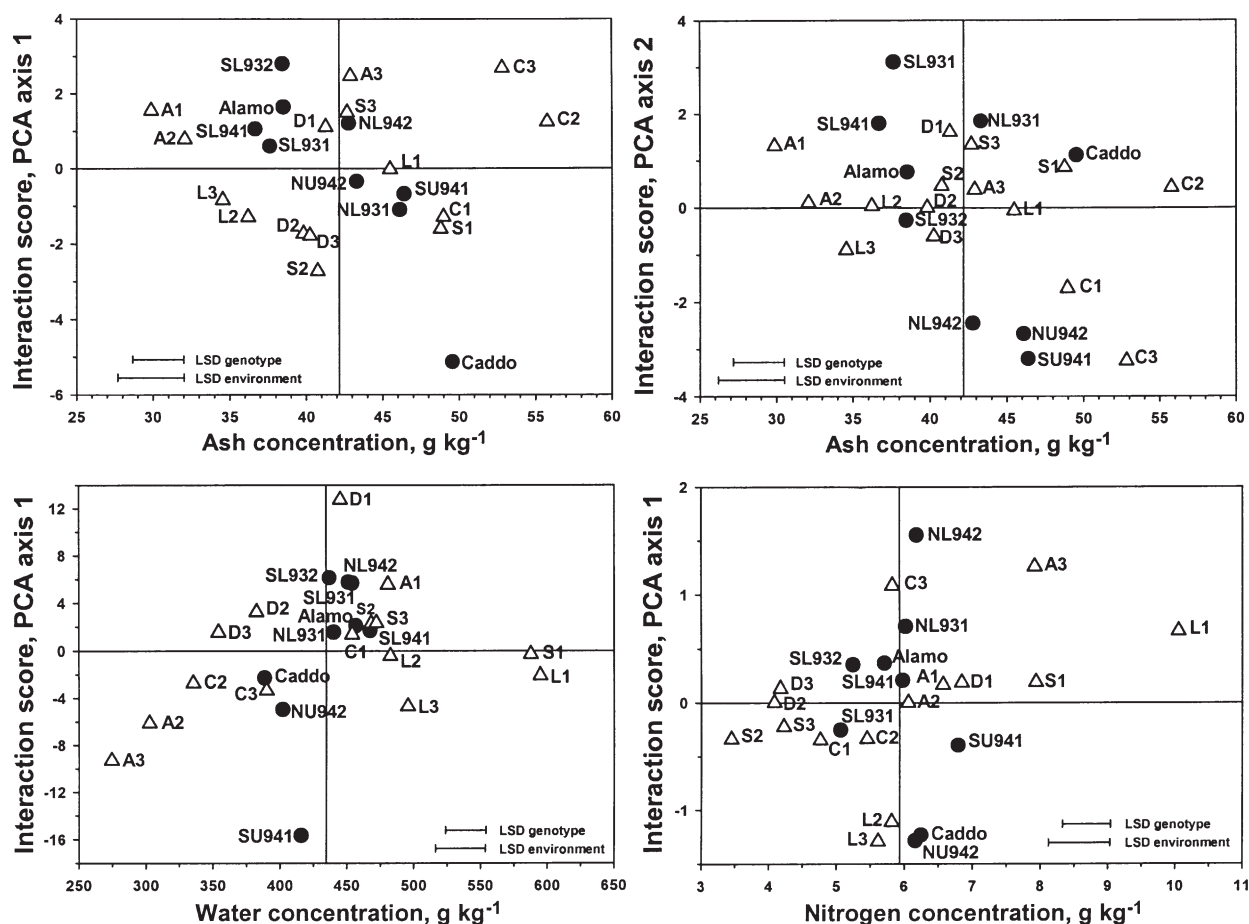

Fig. 6. Biplot of interaction principle component analysis (PCA) axis versus concentrations of ash, N, and water of nine switchgrass genotypes (solid circle) grown over fifteen site–years (open triangle). Site–year labels consist of a site letter (A–Hope, AR; C–College Station, TX, D–Dallas, TX, L–Clinton, LA, and S–Stephenville, TX) plus a year number (1 = 1998, 2 = 1999, 3 = 2000). The vertical line is at the x axis mean.

reducing harvest moisture of grass biofuels to acceptable levels, but at the expense of less harvestable DMY because of crop senescence (Landström et al., 1996; Burvall, 1997; Lewandowski and Kicherer, 1997; Christian et al., 2002). Earlier harvest in late summer would present better drying conditions for baled biomass, but it has the disadvantages of lower yields and higher concentrations of undesirable components like ash and N.

Biomass Ash Concentration

For ash concentration, there were interactions involving all combinations of site, year, and genotype (Table 2) and two significant PCA axes in AMMI analysis (Fig. 6). The second axis showed a strong clustering of ash concentrations for the College Station site. Contrasts (Table 2) showed ash concentrations were greater in upland than in lowland genotypes (48 vs. 40 g kg^{-1}), and higher in northern than in southern ecotypes (45 vs. 40 g kg^{-1}). Ash concentrations were within the range reported for some grass-based biofuel crops including switchgrass (Olanders and Steenari, 1995; Sanderson and Wolf, 1995; Landström et al., 1996; Obernberger et al., 1997; and Lemus et al., 2002), but were generally higher than other reports of ash concentrations for switchgrass (Casler and Boe, 2003), *Miscanthus* (Lewandowski and Kicherer, 1997), and woody feedstocks (Olanders and Steenari, 1995; Obernberger et al., 1997). Individual genotype ash concentrations across all site–years ranged from 24 to 69 mg kg^{-1}, a difference that is large enough to impact fuel heating value (Lewandowski and Kicherer, 1997; Jenkins et al., 1998). The complexity of interactions for this component suggests that multiple factors affected ash concentration. Ash concentration was higher in grass biofuels when the crop was grown on heavy clay soils than when grown on fine sandy soils (Burvall, 1997; Landström et al., 1996). In our trial, ash concentration averaged 40 and 52 g kg^{-1} on a black clay and a silty clay loam (Dallas and College Station, respectively), 38 g kg^{-1} on a silty loam (Louisiana), and 35 and 44 g kg^{-1} on fine sandy loams (Arkansas and Stephenville). Likelihood of soil contamination of biomass is probably also affected by environmental factors such as wind-blown dust, contamination with soil during harvest, and stand characteristics like height, density, or lodging that could influence splashing of soil onto biomass. Lemus et al. (2002) reported that Alamo was lower in ash than most upland genotypes, and that ash concentration of switchgrass was negatively correlated with plant height. This is consistent with our data because upland and northern genotypes are generally shorter in stature than lowland and southern genotypes.

Biomass N Concentration and Soil N Removal Rates

Across site–years (Table 2), contrasts showed that lowland genotypes had lower N concentrations (5.7 vs. 6.3 g kg^{-1}) but higher soil N removal rates (83 vs. 41 kg ha^{-1} yr^{-1}) than upland genotypes, and southern ecotypes had higher soil N removal rates than northern ecotypes (76 vs. 60 kg ha^{-1} yr^{-1}). Nitrogen concentration

of harvested biomass showed interactions between site and year (Table 2) and averaged 6 g kg^{-1} (range 3 to 13 g kg^{-1}). Most individual sample concentrations were well below the critical maximum of 10 g kg^{-1} cited by Lewandowski and Kicherer (1997). Nitrogen removal by the crop (Fig. 4) reflected DMY, averaged 69 kg N ha^{-1} yr^{-1} (range 0 to 214 kg N ha^{-1} yr^{-1}), and was lower than the amount of annually applied fertilizer N for most genotype–site–year combinations. Concentrations of N (Christian et al., 2002; Lemus et al., 2002) and soil–N removal rates (Christian et al., 2002) were within reported ranges for mature or senesced switchgrass biomass.

Biomass P Concentration and Soil P Removal Rates

Phosphorus concentration of harvested biomass showed no pattern in AMMI analysis, nor was it affected by genotype in the ANOVA. Across site–years (Table 2), lowland genotypes had higher P removal rates than upland genotypes (12 vs. 6 kg ha^{-1} yr^{-1}), and southern ecotypes had higher P removal rates than northern ecotypes (11 vs. 8 kg ha^{-1} yr^{-1}). Concentration of P in harvested biomass averaged 0.9 g kg^{-1} (range 0.4 to 1.7 g kg^{-1}), which is similar to concentrations reported for other grass biofuels harvested once yearly at mature or senesced growth stages (Landström et al., 1996; Christian et al., 2002). Biomass P concentration is a minor concern for combustion processes, but there is great interest in crops with the capability to remove P from soils where years of manure application have resulted in accumulation to critical levels. Yearly soil P removal by the switchgrass crop in this trial averaged only 10 kg P ha^{-1} yr^{-1} (range 2 to 27 kg P ha^{-1} yr^{-1}). These removal rates are low in comparison to 70 kg P ha^{-1} yr^{-1} predicted by Pierzynski and Logan (1993) for bermudagrass [*Cynodon dactylon* (L.) Pers.], a prevalent hay crop in the study region. Morris et al. (1982) demonstrated that switchgrass hay P concentration increased with P fertilization, but at a very high fertilization rate of 448 kg P ha^{-1}, forage P was still no more than 2.3 g kg^{-1}. Therefore, use of switchgrass biofuel crops for soil P phytoremediation would be less effective than use of other readily available forages.

CONCLUSIONS

Switchgrass genotypes from different ecotype and morphological type combinations differed in performance across the study region, with genotypes from the southern lowland group consistently yielding the greatest amount of lignocellulose. Variation in DMY had a larger effect on lignocellulose yield than did lignocellulose concentration, with lignocellulose yields paralleling DMY. Therefore, DMY itself was an adequate measure of genotype performance. Latitude had the greatest impact on lignocellulose yields of upland genotypes, but moisture availability and timing also appeared to be important, especially to lowland lignocellulose yields. Moisture, ash, N, and P concentration of harvested biomass were primarily affected by environmen-

tal factors. Biomass moisture concentration at harvest was too high for effective baling or combustion, highlighting a management problem that will require attention before practical adoption of switchgrass for cofiring. Ash concentration was high enough to affect fuel quality, highly variable, and closely related to soil contamination of the harvest. Concentrations of N and P in harvested biomass were low, resulting in relatively low soil nutrient removal rates and confirming the low N and P requirement of switchgrass relative to other biomass crops. Switchgrass biofuel production efforts in the south central region should focus on improving DMY of southern lowland genotypes to maximize lignocellulose yields, but management factors will be more effective in optimizing moisture, ash, and mineral concentrations for combustion.

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